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1. Chien, J. et al. Mol. and Cell. Endocrinology (2001) 181(1-2): 69-79
2. Chien, J. et al. Int. J. of Cancer (2001) 91(1): 46-54
3. Chien, J. et al. Oncogene (1999) 18(22): 3376-3382
4. Wong, E.C.C. et al. Proc. Amer. Assoc. for Cancer Res. (1997) 38: 288
5. Rayford, W. et al. Prostate (1997) 30(3): 160-166
6. Xue-Zhang, Q. et al. Endocrine (1995) 3(6): 445-451
7. Shah, G.V. et al. Endocrinology (1994) 134(2): 596-602
8. Rayford, W. et al. J. of Urology (1994) 151(5 suppl): 490A
9. Rayford, W. et al. J. of Urology (1993) 149(4 suppl): 479A
10. Shah, G.V. et al. Prostate (N.Y.) (1992) 21(2): 87-97
11. Sagol, O. et al. Annals of Medical Sciences (1999) 8(1): 14-21
12. Sussenot, O. et al. Prostate (1998) 36(suppl. 8): 43-51
13. Hanna, F.W. et al. J. Endocrinol. (1997) 152(2): 275-281
14. Sim, S.J. et al. Annals of Clinical and Laboratory Science (1996) 26(6): 487-495
15. Watanabe, K. et al. Fukushima J. Medical Science (1995) 41(2): 141-152
16. Esik, O. et al. European J. Gynaecological Oncology (1994) 15(3): 211-216

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Supplement to The Journal of Urology

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THE ARTIFICIAL NEURAL NETWORK: A NOVEL AND POWERFUL METHOD OF FERTILITY DATA ANALYSIS. C.S. Niederberger, L.I. Lipshultz, and D.J. Lamb, Houston, TX (Presentation to be made by Dr. Niederberger)

Analysis of fertility data is problematic for a variety of reasons: (1) although millions of sperm are ejaculated, the final step in conception requires only one functional sperm; (2) laboratory tests of fertility potential often reflect physiologic mechanisms that interact in complex ways; (3) patient data is often fragmented, and (4) 2 patients are required for the final result. Statistical methods in reproductive medicine are co-opted from other fields, such as oncology, and do not address the unique challenges of fertility data analysis. We are investigating a novel and powerful method of data analysis, the artificial neural network, in the assessment of fertility potential. The neural network is a computational algorithm inspired by the biological structure of the neuron, and "learns" to analyze data according to carefully chosen mathematical rules. To determine the validity of the neural network in analyzing tests of male reproductive function, we programmed a network using a common learning algorithm, backpropagation, to predict the results of sperm penetration assay (SPA) and penetration of sperm in bovine cervical mucus (Penetrak assay, Serono Labs) from the semen analysis (SA). The SPA and Penetrak tests are largely believed to be independent of the SA. Significantly, a neural network correctly predicted the Penetrak result in 80% of the assays that the network had not previously encountered, and another network predicted SPA outcome in nearly 70%. Results were compared to 2 traditional forms of statistical classification, linear and quadratic discriminant function analysis (LDFA and QDFA). The neural network was superior in predicting both assay outcomes. This classification algorithm may prove to serve many of the fundamental needs of data analysis unique to fertility studies.

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SKELETAL MUSCLE WRAP OF THE URINARY BLADDER AND FUNCTIONAL NEUROMUSCULAR ELECTRICAL STIMULATION: DETRUSOR-MYOPLASTY. Michael B. Chancellor¹, Rafael Acosta², Michael J. Erhard¹, David A. Rivas¹, Steven K. Salzman², Philadelphia, PA¹, Wilmington, DE². (Presentation to be made by Dr. Chancellor)

Detrusor-myoelastv (skeletal muscle assisted micturition) was investigated in a rat model of spinal cord injury. Anatomical dissection of the rectus muscle and bladder wrapping in 2 goats and 3 fresh human cadavers was also performed. The rectus muscle on one side of the abdomen was dissected free from the ventral fascia and transected cephalad above the level of the umbilicus. The muscular insertion to the symphysis pubis was left intact. Vascular flow via the inferior epigastric artery and vein was preserved. Innervation by 2 to 3 intercostal motor nerves was left intact.

Postoperatively, no bowel or abdominal wall functional deficit was apparent. The rotated muscular flap remained innervated and vascularized. No difference in 24 hr micturition patterns became evident between control rats and rats with a rectus muscle-wrapped bladder when not stimulated. Stimulation of the rectus muscle-wrapped bladder (both nerve and direct muscle stimulation) in the rat was capable of generating bladder pressure (range 10 to 60 cmH₂O) and achieved bladder emptying. Stimulation parameters ranged from 0.05-0.5 ms duration, 1-50 Hz, and 12.5-300 volts. Less voltage was required for nerve than muscle stimulation to achieve similar intravesical pressure. In both acute experiments and in rats surviving 1 month after spinal cord injury, sustained bladder contractions continued.

Dissection of goats and human cadavers revealed that a vascularized and innervated rectus muscle flap can be rotated into the pelvis and wrapped around the bladder without tension. This is the first report of the principle and technique of detrusor-myoelastv. Detrusor-myoelastv may be applicable for patients with an acontractile detrusor and non-intact sacral motor roots who are not candidates for sacral anterior root neurostimulation.

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EXPRESSION OF NEUROENDOCRINE FACTORS AND EXTRACELLULAR MATRIX DEGRADATIVE ENZYMES IN HUMAN PROSTATE TUMOR CELLS. N.M. Hoosein, C.J. Logothetis, M.G. Bandyk, G.L. Nicolson and L.W.K. Chung, Houston, TX. (presentation to be made by Dr. Hoosein).

Occurrence of neuroendocrine cells in prostate tumors has been associated with tumor progression (Abrahamsson et al., *Pathol. Res. Pract.* 185:373, 1989), early metastatic spread of the disease and poor prognosis (Tetu et al., *Cancer*, 59:1803, 1987; Cohen et al., *Br. J. Urol.*, 66:405, 1990). In this study, we have examined the presence of neuroendocrine substances chromogranin A (ChA), neuron-specific enolase (NSE), bombesin and serotonin in human prostate tumor cell lines and in bone marrow biopsies of prostate cancer patients. Previously, we had found that urokinase-type plasminogen activator (urokinase) plays an important role in determining the invasiveness of human prostate tumor cells (Hoosein et al., *Cancer Commun.* 3:255, 1991). Therefore, we also examined the presence of urokinase as well as another extracellular matrix degradative enzyme, heparanase, (Nakajima et al., *Science* 220:611, 1983) in prostate tumor cells. Results of immunocytochemical staining indicated that neuroendocrine markers ChA, NSE, and serotonin were expressed by prostate tumor cell lines of high metastatic potential, PC-3 and DU-145, but, not by the relatively indolent LNCaP cell line. High levels of bombesin-like immunoreactivity were found in PC-3 and DU-145 cells with lower levels in LNCaP cells. Also, PC-3 and DU-145 cells showed positive staining for urokinase and heparanase, whereas, LNCaP cells were negative for both these enzymes. Nests of tumor cells in bone marrow aspirates of two patients with prostatic adenocarcinoma, stained negative for bombesin, urokinase and heparanase, whilst, those in a patient with small cell carcinoma of the prostate were positive for all three markers. Our results indicate that PC-3 and DU-145 cells display neuroendocrine differentiation and that prostate tumor cells with neuroendocrine characteristics express high levels of degradative enzymes urokinase and heparanase, accounting for their aggressive behavior. Clinically, these neuroendocrine and enzyme markers may serve as valuable predictors of outcome and indicators for aggressive therapy.

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CALCITONIN RECEPTOR AFFECTS INTRACELLULAR CYCLIC 3', 5'-MONOPHOSPHATE (cAMP) LEVELS AND INCREASES CYTOPLASMIC Ca²⁺ CONCENTRATIONS IN HUMAN PROSTATE CANCER CELLS. *Walter Rayford, *Girish V. Shah, *Benjamin J. Frey, Mark J. Noble, Mark Austenfeld, John Weigcl, and Winston K. Mebust, Kansas City, KS (Presentation by Walter Rayford).

Presence of calcitonin (CT)-immunopositive cells have been identified in human prostate and its relative distribution increases in cancer. Our recent findings demonstrate that CT-like immunoreactive material (iCT) is secreted by primary prostate cells in culture, and its secretion from tumor cells is significantly higher than benign cells. Since CT and salmon CT (sCT)-like peptide act on a variety of organs through activation of different, and in some cases, multiple intracellular mechanisms, we have examined 1) whether CT receptors (CT-R) are present in human prostate tissue and human prostate cancer LNCaP cells; 2) the effect of CT on cAMP accumulation and/or cytoplasmic Ca²⁺ concentrations in LNCaP cells.

Surgical prostate specimens and LNCaP cells were homogenized to obtain plasma membrane fractions. The membranes were incubated with various concentrations of [¹²⁵I]-sCT to saturate the binding sites. The data were processed by Scatchard analysis. In a second group of experiments, cultured LNCaP cells were treated with various concentrations of sCT, and their intracellular cAMP levels were analyzed by RIA. In a third group of experiments, LNCaP cells were loaded with Fura 2AM and their intracellular Ca²⁺ levels were analyzed spectrofluorometrically.

Binding studies revealed presence of saturable CT binding sites in human prostate tissues and LNCaP cells. Binding affinity of CT-R in the tissue varied from 1 to 4.3 nM and the B_{max} ranged from 231-925 fmol/mg membrane protein. sCT demonstrated a biphasic effect on cAMP accumulation in LNCaP cells. At concentrations less than 1 nM, sCT significantly attenuated cAMP accumulation with maximal suppression of 30% at 10 pM. However, at concentrations greater than 1 nM, it significantly stimulated cAMP accumulation. The initial results suggest that at 100 nM, sCT significantly increased cytoplasmic Ca²⁺ levels in LNCaP cells. These findings suggest that CT-R is present in human prostate cells, and is coupled to adenylate cyclase and Ca²⁺ second messenger systems. When considered together with its secretion in the prostate, the results suggest a paracrine role for CT in pathophysiology of the prostate.